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Not Freezing Water in the Lamellar System of Chloroplasts

Rolf-Dieter Hirtz and Wilhelm Menke

Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), Köln-Vogelsang

(Z. Naturforsch. 28 c, 230 [1973]; received January 22, 1973)

Chloroplasts, water, PMR-spectroscopy

In the course of small angle X-ray scattering analyses Kreutz and Weber[1] observed that the particles of a soluble protein preparation derived from the lamellar system of chloroplasts by treatment with formic acid, exhibited an unusual high water content of 75 per cent. Viscosimetric investigation by Menke and Ruppel[2] with a preparation obtained by a somewhat modified procedure also pointed to a high hydration of the protein particles. A solution of this protein had a limiting viscosity number $[n]$ of 12 ml/g. With an axis ratio of 1:2.4 of the effective flattened hydrodynamic ellipsoid, also a hydration of 76 per cent was calculated. To our knowledge no investigations have been made on the water content and the state of water in the thylakoid membrane, from which the above mentioned preparations have been obtained. Kuntz, Brassfield, Law, and Purcell[3] observed by means of proton magnetic resonance spectroscopy, that in protein solutions a fraction of the water will not freeze even at —35 °C. The amount of the not freezing water is within the experimental error the same as the amount of hydration water, determined by other methods. It thus appeared obvious to determine with this method the amount of the not freezing water in the lamellar system of chloroplasts.

Stroma-freed chloroplasts of *Antirrhinum majus*, strain 50, were prepared according to an earlier described procedure[4]. The water signals were measured in the HR-mode with external side bands using a Varian HA 100 nuclear resonance spectrometer. For temperature regulation the temperature unit V 6040 was used. The temperature of the sample was determined by temperature depending comparison spectra of methanole. The samples were frozen at —35 °C in the sample holder at the measuring site. The concentration of the stroma-freed chloroplast suspension was 14-19 per cent. The area of the very small water signal, remaining after freezing, was the measure of the amount of not freezing water. These signals were compared with the corresponding signals of bovine serum albumin (Serva cryst., extra pure, > 98 per cent) and of lysozyme (Serva 3 x cryst., extra pure).

The signal area of the chloroplast preparation corresponded at —35 °C to 1.1 ± 0.1 times that of lysozyme and to 1.2 ± 0.1 times the area of bovine serum albumin. For lysozyme Kuntz *et al.*[3] obtained at —35 °C a value of 0.36 g of not freezing water per g protein. The corresponding value for bovine serum albumin was 0.37. Consequently, the stroma-freed chloroplasts contained at —35 °C 0.4 g of not freezing water per g lamellar system or 29 per cent. Hence, the thylakoid membrane and the soluble proteins contain approximately equal amounts of not freezing water. However, the water signal of stroma-freed chloroplasts is broader and lower than the signals of the proteins. From this it should not be necessarily concluded that the not freezing water in the thylakoid membrane is in a different state than in the two proteins. In addition it should be mentioned that not freezing water is not only detectable by proton magnetic resonance but also by means of calorimetry[5,6]. The conclusion from our results is that there is no reason to consider an especially high hydration when building models of the molecular structure of the thylakoid membrane.

We thank Dr. G. H. Schmid for assistance in the preparation of the manuscript.

Requests for reprints should be sent to Prof. Dr. W. Menke, Max-Planck-Institut für Züchtungsforschung, *D-5000 Köln 30.*

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